PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
James H. Prestegard	)	Art Unit: **1631**
	)	
Serial No. **09/880,648**	)	Examiner: **Lori A. CLOW**
	)	
Filed: **June 13, 2001**	)	
	)	
For: **NMR Assisted Design of High Affinity Ligands for Structurally Uncharacterized Proteins**	)	

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S BRIEF UNDER 37 C.F.R. § 1.192

In support of the appeal entered in the above referenced application, Appellant hereby submits this brief under 37 C.F.R. § 1.192.

Real Party in Interest

The real party in interest is the inventor – Dr. James H. Prestegard.

Related Appeals and Interferences.

None.

Status of Claims

Claims 1-4, 6-16 and 19-20 stand finally rejected. Appeal is taken from the rejection of all claims.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450, on August 26, 2005.

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Status of Amendments

Amendments filed after final rejection have not been entered.

Summary of Invention

The invention is a new process for constructing biologically active ligands with improved specificity and binding affinity for biological targets, by starting with a ligand that is known to bind a biological target, identifying a second ligand that binds to the biological target, and constructing a composite ligand from the two ligands in which the distance between the two ligands in the composite ligand, and the orientation of the ligands to one another in the composite ligand, closely mirrors their relative distance and orientation when bound to the substrate as separate ligands. In a principal embodiment, the process is carried out according to the following steps:

1. paramagnetically labeling a ligand that is known to bind to a biological target;
2. preparing a complex between the paramagnetically labeled ligand and the biological target;
3. preparing NMR spectra of the complex;
4. modifying the complex by the addition of a second ligand that binds to the biological target;
5. preparing NMR spectra of the modified complex; and
6. analyzing the spectra to determine whether the second ligand has bound to the biological target within the paramagnetic zone of the label on the first ligand;
7. further analyzing the spectra to determine the distance of separation between the first and second ligand when bound to the biological target;
8. deducing the relative orientation of the first and second ligands when bound to the biological target; and
9. linking the two ligands together at the distance of separation and orientation deduced in steps 8 and 9.

The combined ligands have a greater specificity and affinity for the biological target than either of the ligands by itself.

Issues

The following issues are presented on appeal:

(1) whether the preamble in claims 1 and 19 is unclear because it recites "a method for improving the binding affinity of ligand for a biological target," when the claim does not specifically recite an improvement in the binding affinity.

(2) whether step (c) of claim 20 is rendered unclear by the presence of the term "substantially."

(3) whether the claims are properly rejected as obvious over the combined teachings of Johnson et al. (1999) and Bolon et al. (1999).

Argument

Claim Rejections – 35 U.S.C. § 112, second paragraph

The Final Office Action rejects claims 1 and 19 because they recite a method for improving the binding affinity of a ligand for a target and do not provide a step of improving the binding affinity. This rejection is improper because (1) the objectionable limitation is found in the preamble, which does not even limit the claim, and (2) the binding affinity is inherently improved in a significant number of constructs whenever one carries out the recited sequence of steps.

The Final Office Action rejects claim 20 because clause (c) contains the term "substantially." Applicant respectfully submits that a skilled worker would understand precisely what is meant by the term substantially, since it refers to a distance and orientation that improves the binding affinity of the resulting compound.

Claim Rejections – 35 U.S.C. § 103

The Final Office Action also rejects the claims as obvious based on the combined teachings of Johnson (1999) and Bolon (1999). During a telephone interview conducted on March 15, 2005, the Examiner informed Applicant's attorney that she was relying on page 620, in column 2 of Johnson, which describes a composite protein that Johnson constructed by linking together two cellulose binding domains (CBD_{N1} and CBD_{N2}) of the CenC beta-1,4-glucanase enzyme. This linking of two protein domains is in contrast to the linking of two parts of a ligand that binds to a protein as described in the instant claims. Johnson was studying the composite

protein with the two CBDs to gain some understanding of the binding characteristics of the CBDs to cellulose, but ultimately he concluded that the relative orientation of the CBD protein domains did not even matter, because "the tandem CBDs anchor CenC to its natural substrate ... without a strong preference for their orientations." (P. 620 at col. 2.)

Johnson did not link the CBD domains of the composite protein at a preferred orientation and distance to improve their binding affinity for a ligand, or undertake any steps to characterize that preferred distance and orientation, all as required by the claims of the present invention. He does not identify the structure of the linker, the distance between the two CBDs when bound to the linker, or the relative orientation of the two CBDs when bound to the linker. In fact, he states just the opposite in column 2 of page 620 when he states that "the spatial arrangement of these modules (i.e. the two CBD ligands) within native C. *fimi* CenC remains to be defined." Continuing in the second column of page 620, Johnson further confirms the point when he states that the two ligands "cannot bind simultaneously to adjacent regions of a single polymer chain" due to "structural constraints" in the linker.

Johnson never addressed the issue of a distance between a first and second ligand and never "determine[ed] whether a second ligand perturbs peaks on the second NMR spectra that are also perturbed by the paramagnetic label on the first NMR spectra," as required by clause (d) of claim 1. That step would only be performed if one was interested in determining whether the two ligands bound to the target molecule at an ascertainable distance, and constructing a composite ligand at the distance ascertained. Moreover, no orientational data from dipolar couplings were collected as described in claims i.e. 7, 19(e) and 20(b). The "orientation" of the cellulose-derived ligand recited by Johnson actually refers to a "direction" of binding in the protein site which proves to be ambiguous, as opposed to a three dimensional orientation. This latter information was also dependent on use of assigned protein resonances, a step not needed in the present invention.

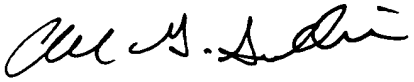
To overcome these deficiencies, the Examiner makes reference to the fact that Johnson reports some spin label studies that he conducted in column 1 of page 620. These studies were performed to locate the binding site of a single ligand on the protein surface, not to define distances between two ligands; again it required assignment of protein resonances. Once again, this represents a fundamental difference between the method Johnson was employing and the method described in the instant claims.

The Examiner also cites to Bolon (1999) to cure these deficiencies, but Bolon merely reports the availability of residual dipolar couplings to deduce the relative orientation of a ligand when bound to a biological target based upon observations of residual dipolar couplings. The article only analyzed the orientation of a **single** ligand relative to a protein structure. The authors did not bind two different ligands to the protein structure -- as required by the pending claims -- , deduce the relative orientations of the two ligands – as required by the pending claims -- or determine the distance between the ligands – again as required by the pending claims, because he was not concerned with improving the binding affinity of a ligand for the biological target by making composite ligands. In short, the reference did not offer any suggestion or motivation for constructing composite ligands based upon their relative distances and orientations when bound to a protein, as recited in the pending claim. Lacking such a suggestion or other motivation, the references do not support a prima facie case of obviousness.

CONCLUSION

In view of the arguments presented herein, Applicants respectfully request that the final rejection in this matter be vacated, and that this application be returned to the examiner with instructions to enter a notice of allowance.

Respectfully submitted,



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APPENDIX 1

LISTING OF PENDING CLAIMS APPEALED FROM

1) A method for improving the binding affinity of a ligand for a biological target comprising:
   a) preparing first NMR spectra of a first complex comprising the biological target and a paramagnetically labeled derivative of a first ligand to define a paramagnetic zone;
   b) preparing second NMR spectra of a second complex comprising the biological target and a second ligand;
   c) analyzing the spectra to determine whether the second ligand binds to the biological target within the paramagnetic zone of the paramagnetically labeled derivative;
   d) deducing a relative three-dimensional orientation of the first and second ligands when bound to the biological target;
   e) deducing a distance of separation of the first and second ligands when bound to the biological target; and
   f) selecting or preparing a compound that contains the first and second ligands substantially in the relative orientation and distance.
2) The method of claim 1 wherein step (c) is performed by:
   a) identifying peaks on the first NMR spectra that are perturbed by the paramagnetic label; and
   b) determining whether the second ligand perturbs peaks on the second NMR spectra that are also perturbed by the paramagnetic label.
3) The method of claim 1 wherein the first complex further comprises the second ligand, and step (c) is performed by determining whether the paramagnetically labeled derivative of the first ligand perturbs peaks associated with the second ligand.
4) The method of claim 3 further comprising, before step (c), preparing third NMR spectra of a mixture of the paramagnetically labeled derivative of the first ligand and the second ligand in the absence of the biological target.
5) (CANCELLED)
6) The method of claim 1 wherein the distance of separation is determined as a function of the loss of intensity for NMR resonances from the second ligand.

7) The method of claim 1 wherein the three dimensional orientation is deduced by producing a field ordered state in a medium comprising the biological target, the first ligand, and the second ligand, and analyzing dipolar couplings within the first and second ligands.

8) The method of claim 7 wherein the field ordered state is produced by an aqueous dispersion of lipid bicelles having complementary charges to the biological target.

9) The method of claim 7 wherein the field ordered state is produced by an aqueous dispersion of bacteriophage having a domain of the biological target in the outer coat.

10) The method of claim 1 wherein the paramagnetic label is a nitroxide or metal chelate.

11) The method of claim 1 wherein the first and second NMR spectra are two dimensional heteronuclear single quantum coherence spectra.

12) The method of claim 1 wherein the biological target is isotopically labeled.

13) The method of claim 1 wherein the biological target is a protein, and NMR resonances from the protein are not assigned to a sequence of the protein.

14) The method of claim 1 wherein the biological target is a protein, and the three dimensional conformation of the protein is unknown.

15) The method of claim 1 wherein the first ligand is an oligosaccharide, and the biological target is a protein.

16) The method of claim 1 wherein the second complex comprises the first ligand, or a paramagnetically labeled derivative thereof.

17) (CANCELLED)

18) (CANCELLED)

19) A method for improving the binding affinity of ligands for biological targets comprising:

a) preparing first NMR spectra of a first complex comprising a biological target, a paramagnetically labeled derivative of a first ligand, and a second ligand;

b) preparing second NMR spectra of a second complex comprising the biological target and either the second ligand or the paramagnetically labeled derivative of the first ligand;

c) preparing third NMR spectra of a mixture of the paramagnetically labeled derivative of the first ligand and the second ligand in the absence of the biological target;

d) analyzing the spectra to determine whether the paramagnetically labeled derivative of the first ligand perturbs peaks associated with the second ligand;

e) deducing a relative three-dimensional orientation of the first and second ligands when bound to the biological target;

f) deducing a distance of separation of the first and second ligands when bound to the biological target; and

g) selecting or preparing a compound that contains the first and second ligands substantially in the relative orientation and distance.

20) The method of claim 19 further comprising:

a) deducing from the NMR spectra the distance between the first and second ligands when bound to the biological target;

b) deducing from the NMR spectra the relative three dimensional orientation of the first and second ligand when bound to the biological target; and

c) selecting or preparing a hybrid ligand that contains the first and second ligands covalently linked substantially at the bond distance and relative orientation deduced in steps (a) and (b).